The Cytokine Handbook

Second Edition

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Chapter 12

Interleukin-10

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INTRODUCTION

IL-10 was originally discovered during a search for a cross-regulatory cytokine that would be produced by T_{H2} cells and inhibit the functions of T_{H1} cells. We found that T_{H2} supernatants contained an activity that inhibited cytokine production in cocultures of T_{H1} cells, antigen-presenting cells (APCs) and antigen (Fiorentino *et al.*, 1989). This effect was specific for T_{H1} cells since T_{H2} cells responded normally in the presence or absence of the T_{H2} supernatant factor, which was named cytokine synthesis inhibitory factor (CSIF). After immunochemical and biochemical analysis indicated that CSIF was likely to be a novel cytokine, a cDNA clone encoding CSIF was isolated by expression cloning (Moore *et al.*, 1990). Characterization of the recombinant cytokine revealed that additional activities of CSIF were being analysed in other laboratories. These activities included stimulation of proliferation of mast cells (Thompson Snipes *et al.*, 1991) and thymocytes (Suda *et al.*, 1990). The name 'Interleukin-10' was then proposed (Moore *et al.*, 1990). The mouse cDNA sequence was used to isolate a human homologue from a human T-cell cDNA library (Vieira *et al.*, 1991), and the biological activities of the human recombinant IL-10 were found to be similar to those of the mouse cytokine. As observed for many other cytokines, IL-10 mediates several functions on multiple cell types. IL-10 inhibits several macrophage functions, including presentation of antigen to T_{H1} cells, cytokine synthesis and some microbicidal activities. In contrast, IL-10 generally enhances or stimulates mast cells and B cells. IL-10 is produced by macrophages and other cell types, in addition to the T cells from which it was originally identified, and so IL-10, in common with several other cytokines, has a much more complex role in the immune system than could be inferred from the original activity.

BIOCHEMICAL PROPERTIES OF IL-10

Mouse IL-10 has an approximate molecular mass of 35 kDa (Fiorentino *et al.*, 1989) and is a non-disulphide-linked homodimer. The monomer polypeptide chains migrate during SDS–gel electrophoresis in two major bands corresponding to apparent molecular masses of 17 kDa and 21 kDa. Treatment with N-glycanase, or synthesis in the presence of tunicamycin, results in nonglycosylated IL-10 that migrates at 17 kDa

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Table 1. Functions of IL-10.

Macrophages
Inhibition of cytokine production in response to LPS and T cells (IL-1, IL-6, IL-8, IL-10, IL-12, TNF)
Inhibition of NO production
Reduction of APC function for T_{H1} cells.
NK cells
Inhibition of cytokine production
T cells
Inhibition of cytokine synthesis by T_{H1} and CD8 cells (indirect, when macrophages are APC).
Enhancement of mouse CTL differentiation
B cells
Enhancement of proliferation (human).
Enhancement of antibody secretion (human)
Enhancement of MHC II expression (mouse)
Mast cells
Enhancement of proliferation
Enhancement of protease expression
In vivo
Inhibition of DTH induction
Inhibition of DTH effector function

(Moore *et al.*, 1990). In contrast, human IL-10 has little or no glycosylation and migrates as a single band at about 18 kDa. Glycosylated and nonglycosylated mouse IL-10 appear to have similar activities, at least *in vitro*. Chromatography on a hydrophic interaction column separates three components, corresponding to glycosylation of two, one or neither of the polypeptides. All three species have similar specific bioactivities (Bond, Fiorentino and Mosmann, unpublished data). Mouse and human IL-10 are very labile in acid solutions and activity is lost rapidly below pH 5.5. Monoclonal antibodies specific for mouse IL-10 revealed that, as for many other cytokines, some IL-10 molecules appear to be nonfunctional and antigenically different, since two monoclonal antibodies were isolated that bound IL-10 but did not recognize any biologically active molecules (Mosmann *et al.*, 1990). Four other monoclonal antibodies recognized active IL-10, but did not cross-absorb the IL-10 molecules recognized by the other two antibodies.

RECOMBINANT CLONING OF IL-10

An IL-10 cDNA clone was isolated by expression cloning. Pools of a cDNA library from an activated T_{H2} clone (D10) in the pcDSR-α cloning vector (Takebe *et al.*, 1988) were screened for their ability to direct the synthesis of CSIF activity in COS cells. A full-length cDNA clone encoding CSIF activity was isolated, and the sequence of the open reading frame was not related to any of the known cytokines. A cDNA clone for human IL-10 was isolated from a human T-cell cDNA library by cross-hybridization with mouse IL-10 oligonucleotide probes (Vieira *et al.*, 1991). Conserved regions of the mouse and human clones were used to design PCR primers for the amplification of rat IL-10 cDNA from RNA extracted from Con-A-stimulated T cells from a parasite-infected rat (Goodman *et al.*, 1992). The amplified product was then cloned. Human